INDICATOR BASED AND INDICATOR – FREE ELECTROCHEMICAL DNA BIOSENSORS

Kagan KERMAN, Burcu MERIC, Dilsat OZKAN, Pinar KARA and Mehmet OZSOZ*
Ege University, Faculty of Pharmacy, Dept. of Analytical Chemistry,
35100, Bornova, Izmir - TURKEY
*Author to whom correspondence should be sent :
ozsozs@pharm.ege.edu.tr

INTRODUCTION

Nucleic acids have become the ultimate tools in the recognition and monitoring of many important compounds [1]. There is a great demand for a detection system which cannot only determine specific DNA fragments, but can also determine the exact total nucleic acid content of a sample. For more than a decade, DNA biosensor technologies are under intense investigation owing to their great promise for rapid and low-cost detection of specific DNA sequences in human, viral and bacterial nucleic acids [2].

Recently, some electrochemical techniques that are well suited for detecting hybridization and DNA damage events have been reported. Hybridization can be detected by redox-active metal complexes that associate selectively and reversibly with double stranded immobilized DNA [3,4]. Erdem et al. [5,6] reported that methylene blue (MB) could be used as a redox-active indicator for the electrochemical detection of mismatched bases in DNA.

Only a little attention has been paid to the development of electrochemical DNA biosensors that do not require an external redox indicator. Wang et al. [7] described an indicator-free electrochemical DNA biosensor protocol, which involves the immobilization of inosine-substituted (guanine-free) probe onto CPE and the detection of hybrid formation was performed by using the appearance of the guanine oxidation signal of the target in connection with chronopotentiometric stripping analysis (PSA). Napier et al. [8] also used inosine substituted probes, which still recognized cytidine but not donated an electron to $[Ru(bpy)_3]^{2+}$. Their hybridization indicator, $[Ru(bpy)_3]^{2+}$, exhibited a reversible redox couple at 1.05 V and oxidized guanine in the products of polymerase chain reaction (PCR) of genomic material from infectious organisms.

Methylene blue (MB) is an aromatic heterocycle that binds strongly to DNA via intercalation. MB interacts in a different way with ssDNA and dsDNA. The decreased electrochemical response due to the association of the large planar hydrophobic phenothiazine group with the surface duplex thus serves as the DNA recognition signal [5,6]. In particular, the cationic charge of MB would improve the DNA binding affinity electrostatic interaction with phosphate backbone. Barton and co-workers [9] reported on a gold electrode derivatized with DNA oligonucleotides containing a pendant 5'-hexanethiol linker. They used this electrode to detect the point mutations in DNA by using the electrocatalyzis of the MB, which is intercalated into the duplex at the electrode surface.

Here we describe an electrochemical DNA biosensor for the detection of DNA hybridization using the oxidation signals of guanine and adenine and the reduction signals of MB in connection with DPV.

EXPERIMENTAL SECTION

Apparatus

The oxidation signals of adenine and guanine were investigated by using DPV with an AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.8 software package (Eco Chemie, The Netherlands). The three electrode system, consisted of the in-house made carbon paste electrode (CPE) as the working electrode, the reference electrode (Ag/AgCl) and a platinum wire as the auxiliary electrode. The body of CPE was a glass tube (3 mm i.d.) tightly packed with the carbon paste. The electrical contact was provided by a copper wire inserted into the carbon paste. Carbon paste was prepared in the usual way by hand-mixing graphite powder (Fisher) and mineral oil (Acheson 38) in a 70:30 mass ratio. The surface was polished on a weighing paper to a smoothed finish before use. The convective transport was provided by a magnetic stirrer.

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25 Oct 2001	N/A	-

Title and Subtitle Indicator Based and Indicator - Free Electrochemical DNA Biosensors	**Contract Number**
	Grant Number
	Program Element Number
Author(s)	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) Ege University Faculty of Pharmacy Izmir - Turkey	**Performing Organization Report Number**
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	**Sponsor/Monitor's Acronym(s)**
	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Oct 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom, The original document contains color images.

Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** UU

Number of Pages
4

Chemicals

Double stranded calf thymus DNA (dsDNA, activated and lyophilized) and single stranded calf thymus DNA (ssDNA, activated and lyophilized) were purchased from Sigma. The 17-base synthetic oligonucleotides were purchased from Synthegen, LLC (Houston, Texas, USA); their base sequences are as below:

target (17-base sequence A):

5′-TAA-GCA-ACC-TGA-TTT-GA-**3′**

immobilized probe (17-base sequence B):

5′-TCA-AAT-CAG-GTT-GCT-TA-**3′**

Two - bases mismatch (17-base sequence A′):

5′ - TAA-GCA-A**GG**-TGA-TTT-GA- **3′**

The 17-base sequence A is complementary to 17-base sequence B; 17-base sequence A′ is a mutant of the 17-base sequence A with two-base changed, as indicated by the underlines.

RESULTS AND DISCUSSION

The DNA biosensor relied on the electrochemical transduction of the hybridization between the probe and complementary sequences. The detection of hybridization was accomplished by using the oxidation signals of guanine and adenine, where the electroactivity of these bases led to significantly enhanced voltammetric signals. The decrease in the magnitude of the voltammetric peaks of guanine and adenine thus reflected the extent of the hybrid formation. The detection method therefore involves monitoring the intrinsic signals of DNA by DPV, which is enhanced in the presense of probe due to the higher oxidation signals of free guanine and adenine.

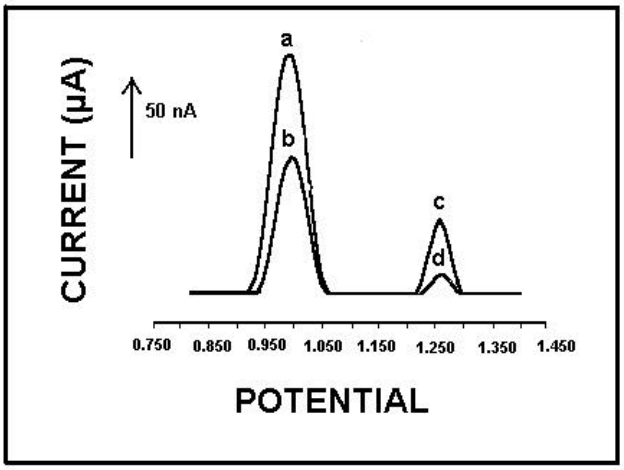

Figure 1.

The changes in DPV peak currents of guanine and adenine obtained with the ssDNA and dsDNA modified CPEs are shown in Figure 1. The oxidation peak potentials of the guanine (Figure 1a and 1b) and adenine (Figure 1c and d) were found at around 1 V and 1.20 V, respectively, in both electrodes. The signals obtained with the ssDNA modified CPE (Figure 1a and Figure 1c) were higher than the ones obtained with the dsDNA modified CPE (Figure 1c and d). Due to the binding of guanine and adenine bases to complementary cytosine and thymine bases in dsDNA, the redox active groups of guanine and adenine were only partly avaliable for oxidation; and the peak current observed for them at the dsDNA modified CPE decreased to about 51.6 %.

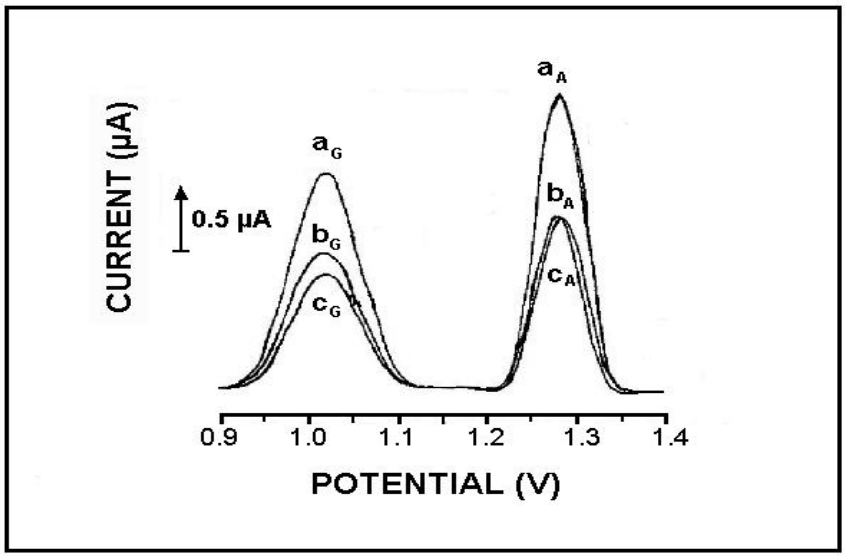

Figure 2.

Sequence-specific hybridization experiments were performed to assess whether the probe-modified CPE responded selectively to the target (Figure 2). High oxidation signals of guanine (Figure $2a_G$) and adenine (Figure $2a_A$) were obtained from the probe modifed CPE. Hybrid formation on the CPE with the target sequence resulted in the decrease of the guanine (Figure $2c_G$) and adenine (Figure $2c_G$) signals. The response of the two-bases mismatch oligonucleotide at the DNA biosensor was also detected by using the intrinsic adenine and guanine signals (Figure $2b_G$). In the presence of an oligonucleotide containing a two-bases mismatch (sequence A') which were guanine bases nearly in the middle of this sequence, the difference between the signal of the sequence A - sequence B hybridization and the one of the sequence A' - sequence B hybridization could be observed. This difference indicated that the complete hybridization was not accomplished. No difference in the adenine signals were observed from the hybridization between probe and mismatch containing sequences (Figure $2b_A$). Since the mismatched bases were guanines, the increase in the signal of the sequence A' - sequence B hybridization assumed to be based on the presence of unbound guanine bases available for oxidation (Figure $2b_G$).

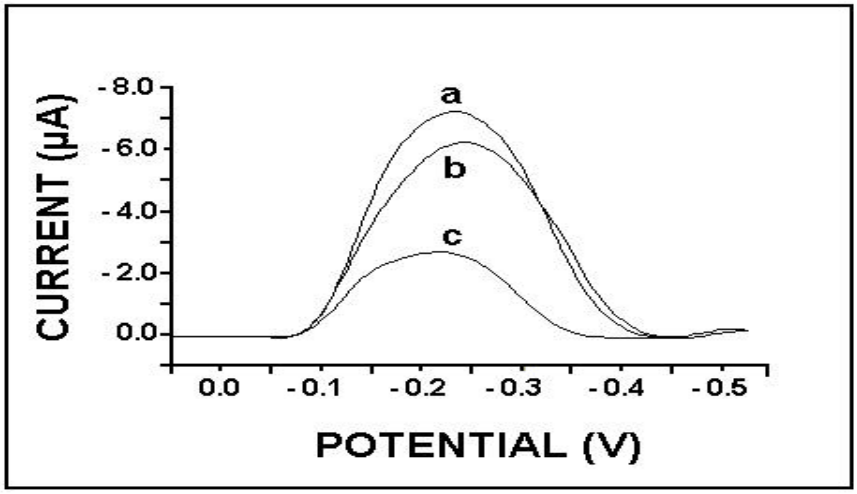

Figure 3.

Figure 3 shows differantial pulse voltammograms for MB at the 17-base probe - modified CPE (a) before hybridization, (b) after hybridization with the two-bases mismatch oligonucleotide (sequence C), (c) after hybridization with 17-base Microcystis spp. target (sequence A). A significant increase in the voltammetric peaks are observed for the indicators, showing that MB has reversible electroactivity and strong association with the guanine residues on immobilized probe (Figure 3a). Figure 3b shows the substantial response of the two-bases mismatch oligonucleotide at the DNA biosensor is also detected by using MB. In the presence of an oligonucleotide containing an two-bases mismatch (sequence A') which included guanine bases nearly at the middle of this sequence, the difference between the signal of the sequence A - sequence B hybridization and the one of the sequence A' - sequence B hybridization could be observed. MB reported here is capable of selectively discriminating against mismatches, as desired for the detection of disease-related point mutation in the guanine bases of the cyanobacteria. The resulting biosensors offer great promise for mismatch-sensitive hybridization detection and can operate over a wide range of hybridization conditions.

CONCLUSION

The utility and advantages of an indicator free and MB based sequence specific DNA hybridization biosensor based on guanine and adenine oxidation signals and MB reduction signals have been demonstrated. Indicator free detection system is simple, cost-effective and provides rapid detection. The application of DPV at CPE fulfilled the expectations for the direct detection of hybridization between the known oligonucleotides. This procedure can be employed to detect specific gene sequences related to different viruses, bacterias or even inherited diseases. Improving the immobilization and hybridization steps through the use of covalent attachment schemes or PNA probes should futher minimize non-specific adsorption effects and maximize sensitivity and speed. Reports are in progress towards these directions.

REFERENCES:

[1] E. Palecek, M. Fojta, Detecting DNA hybridization and damage, Anal. Chem. 73 (2001) 75A-83A.

[2] J. Wang, From DNA biosensors to gene chips, Nucl. Acids Res. 28 (2000) 3011-3016.

[3] A. Erdem, K. Kerman, B. Meric, U. S. Akarca, M. Ozsoz, DNA Electrochemical Biosensor For The Detection of Short DNA Sequences Related To The Hepatitis B Virus, Electroanal. 11 (1999) 586-588.

[4] A. Erdem, B. Meric, K. Kerman, T. Dalbasti, M. Ozsoz, Detection of Interaction Between Metal Complex Indicator and DNA by Using Electrochemical Biosensor, Electroanal. 11 (1999) 1372-1376.

[5] A. Erdem, K. Kerman, B. Meric, U. S. Akarca, M. Ozsoz, A Novel Hybridization Indicator Methylene Blue for the Electrochemical Detection of Short DNA Sequences Related to the Hepatitis B Virus, Anal. Chim. Acta 422 (2000) 139-149.

[6] A. Erdem, K. Kerman, B. Meric, M. Ozsoz, Methylene Blue as a Novel Electrochemical Hybridization Indicator, Electroanal. 13 (2001) 219-223.

[7] J. Wang, G. Rivas, J. R. Fernandes, J. L. L. Paz, M. Jiang, R. Waymire, Indicator-free electrochemical DNA hybridization biosensor, anal. Chim. Acta 375 (1998) 197-203.

[8] M. E. Napier, C. R. Loomis, M. F. Sistare, J. Kim, A. E. Eckhardt, H. H. Thorp, Probing biomolecule recognition with electron transfer : Electrochemical sensors for DNA hybridization, Bioconjugate Chem. 8 (1997) 906-913.

[9] E. M. Boon, D. M. Ceres, T. G. Drummond, M. G. Hill, J. K. Barton, Mutation detection by electrocatalysis at DNA-modified electrodes, Nature Biotech. 18 (2000) 1096-1100.